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An HPLC Method for Identification and Separation of some Phenolic Acids in the Coffee

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Abstract

A simple, reproducible and sensitive HPLC method for determination of caffeic acid, p-coumaric acid, ferulic acid and o-coumaric acid in roasted coffee samples has been developed. The phenolic acids were separated using Kromasil 100-5C18 column by gradient elution with a flow rate of 0.9 ml min^{-1}. The samples were monitored at 320 nm for all phenolic acid using photo-diode array detection. The liniarity, selectivity, system performance parameters, precision had acceptable values. This method may be useful to differentiate and to characterize the types of coffee according to their geographical origin.

Key words*: Phenolic acids, coffee, HPLC*

Introduction

The coffee plant belongs to the genus of Coffea (Rubiaceae family). Coffee is a giant global industry and ranks second only to petroleum in terms of dollars traded worldwide. Presently, coffee production is about 6.3 milion tons, with Brazil and Columbia contributing to nearly 44% of these figures. [1]

Coffee is the most consumed food product in the world. Brazil is the main producer, and exporter, beeing responsible for 35% of world production. Brazilian coffee is present in most bends sold around the world. Moreover, Brazilian internal coffee consumption has been growing significantly in recent years. Today, Brazilian consumption reprezents 14% of world coffee demand and corespond to 32% of the whole European coffee market. [2]

Phenolic compound are ubiquitous constituents of higher plants found in the wide range of commonly consumed plants foods such as fruits, vegetables, cereals and legumes, and in beverages of plant origins, such as wine, tea and coffee. These compounds are secondary metabolites of plants generally involved in defence against ultraviolet radiation or aggression by patogens. Most of these compounds have received considerable attention as potentially protective factors against human chronic degenerative diseases (cataracts, neurodegenerative diseases), cancer and cardiovascular disease.

While condensed tannins are the main phenolic compounds in the coffee pulp, in the seed, phenolic compounds are present predominantly as a family of esters formed between certain hydroxycinnamic acids and quinic acid, collectively known as chlorogenic acids (CGA).[3].

Chlorogenic acids (CGA) are a group of compounds well represented in coffee beans (5-10%). Chemically, CGAs comprise a group of esters of quinic acid with some specific phenols, mainly caffeic, ferulic, and p-coumaric acids known as hydroxycinnamic acids (HCAs). The real contribution of these compounds to flavor in roasted coffee and the real importance in coffee quality remains not well understood. By enzymatic or alkaline hydrolysis, HCAs can be obtained from endogenous CGAs in green coffee and can be of importance in its quality control.

Monitoring hydroxycinnamic acids in coffee can be useful, particularly for the coffee industry, both for assessing their levels in raw materials before and after roasting and also to help the definition of authenticity of the commercial coffee varieties and for a possible characterisation of their geographical origin. However, the methodologies for individual HCAs are quite scarce in literature; thus an adequate methodology is required. [4]

The purpose of this article, is to describe an adequate, simple, reproducible, technique for simultaneous determination of four phenolic acids in roasted coffee sample: caffeic, p-coumaric, ferulic, o-coumaric.

Experimental

Reagents

Formic acid, ethyl acetate, methanol were all of analytical or HPLC grade and purchased from Merck and Scharlau. All phenolic acids standards (caffeic, p-coumaric, ferulic, ortho-coumaric) were obtain from Sigma/Fluka.

Sample preparation

Arabica roasted coffee samples were purchased from local store.

Extraction condition were the same as those reported by Andrade at al.[4]. A 3 g portion of coffee samples, finely powdered, was blended with 60 mL of methanol/water (40/60) during 24h. The mixture was filtered and the filtrate concentrated under vacuum (40°C) to a volume of 5 mL. This solution was hydrolysed with 5 mL of 2N NaOH for 240 min. The pH of the mixture was adjusted to pH 7.00 with 2N HCl and the phenolic acids were extracted by liquid/liquid extraction with ethyl acetate (20mLx3). The extracts were then combined and the ethyl acetate removed under reduced pressure. The residue was dissolved in 7 mL of methanol and 10 µl were analysed by HPLC.

Instrumentation and chromatographic conditions

The separation of phenolic acids was performed with an LC system consisting of an Agilent 1100 series quaternary pump whith a degasser and a photo-diode array detector.
The samples where injected to an HP Agilent 1100 autosampler with a termostatted column compartment on a Kromasil 100-5C18 (250 mm×4.6 mm, 5µm) column at 25 ^{0}C. The system was controlled and data analysis was performed with Agilent ChemStation software. All the calculation concerning the quantitative analysis where performed by external standardisation by measurement of the peak areas.

Resolution of the 4 phenolic acids and subsequent analytical investigations were carried out with the Kromasil 100-5C18 (250 mm × 4.6 mm, 5µm) using methanol (B) and aqueous formic acid (A) as the binary solvent system. The flow rate was set at 0.9 ml/min. Column and guard column were thermostatically controlled at 25^{0}C. Injection volume was set to 10 µl.

Quantification

Stock and Standard Solutions

Nearly 50 mg of each phenolic acid (caffeic acid, p-coumaric acid, ferulic acid and o-coumaric acid) where accurately weighed and dissolved into a 100 mL volumetric flask, in methanol, and filled up to volume for preparing stock solutions. Standard solutions were prepared in methanol for each phenolic acid al least five different concentration level in a 10 ml volumetric flasks.

Chromatographic Conditions

HPLC analysis was performed by gradient elutions at a flow rate of 0.9 ml/min. The mobile phase was delivered from two separate containers with gradient elution program. The first container was water - formic acid (19:1) (solution A) and the second container was methanol (solution B). All solvents were filtered to a 0.45 um Milipore filter prior to use and degassed in a ultrasonic bath. A gradient system were used as follows:

Table1. Gradient elution program

Time	**A**	**B**	**Flow rate**
min	%	%	ml/ min
0	70	30	0.9
20	50	50	0.9
25	70	30	0.9

Ten microliter volumes of each standard solution and sample were injected into the column and the chromatograms were recorded at 320 nm.

Results and discussion

Linearity

The external standard method was the technique used for quantitation. Peak areas from HPLC chromatogram were plotted against the known concentrations of stock solutions of varying concentrations.

Under the assay conditions described, a linear relationship between the concentration of phenolic acids and the UV absorbance at 320 nm was obtained. The correlation coefficient for each standard curve invariably exceeded 0.99 for all phenolic acids.

Table 2 presents the equation of the regression line, correlation coefficient, and the intercept for each compound.

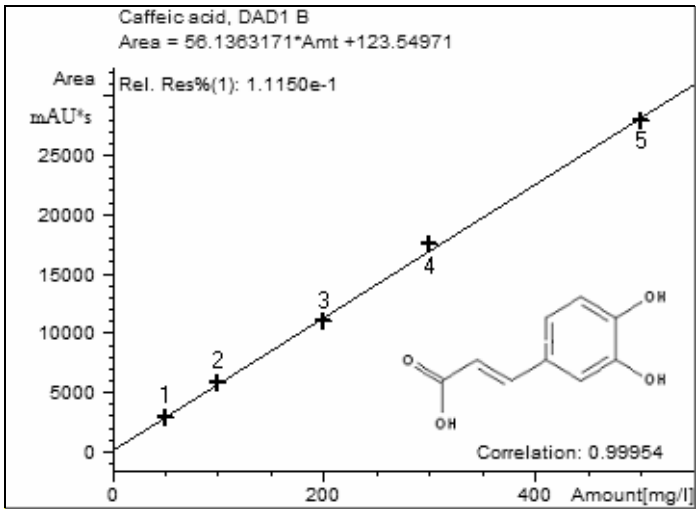

Fig. 1. Caffeic acid calibration curve

Table 2. Linearity results, limit of detection (LOD) and limit of quantification (LOQ)

Compound	Equation	Correlation	LOQ (mg/L)	LOD (mg/L)
Caffeic acid	Y = 56.1363171X+123.54971	0.99954	0.199	0.066
p-coumaric acid	Y = 66.6846225X +183.09358	0.99961	0.178	0.059
Ferulic acid	Y = 48.8189547X+1433.7753	0.99168	0.477	0.157
o-coumaric acid	Y = 27.4615666X+80.517938	0.99987	0.076	0.025

Limit of Detection and Quantification

The detection limits value was calculated based on standard deviation of the response and the slope of the calibration curves.

The LOD was calculated as 0.066, 0.059, 0.157 and 0.025 mg/L for cafeic acid, p-coumaric acid, ferulic acid and o-coumaric acid respectively (Table 2).

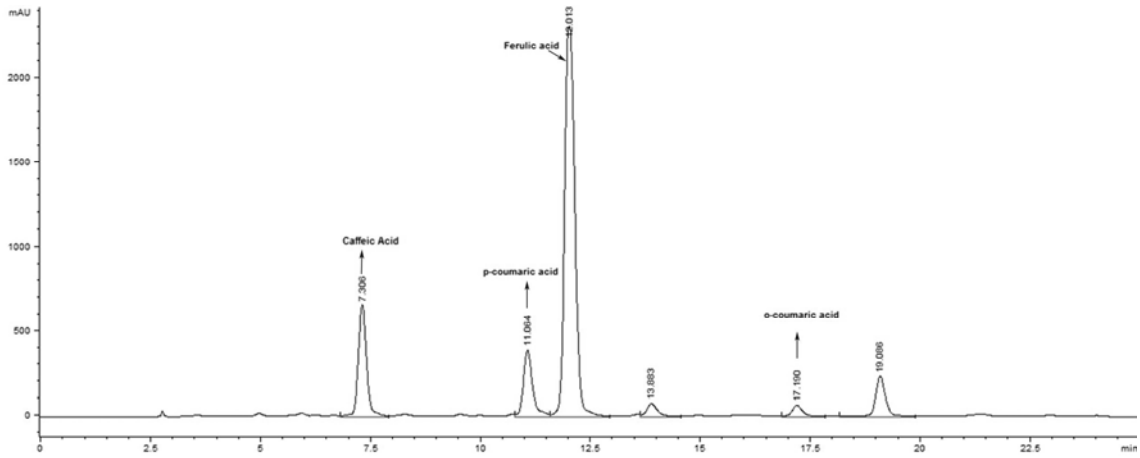

Fig. 2. HPLC phenolic acids profile of a roasted coffee sample
Detection at 320 nm. (1) Caffeic acid; (2) p-coumaric acid; (3)Ferulic acid; (4)o-coumaric acid;

The retention times (RT) obtained for phenolic acids were: RT 7.3 minute for caffeic acid, RT 11.06 minute for p-coumaric acid, RT 12.01 minute for ferulic acid, RT 17.19 minute for o-coumaric acid.

As an example, Figure 2 shows the HPLC phenolic acids profile of a arabica coffee sample. The unidentified compounds recorded with diode array-detector with maximum at 320 nm could be hydroxycinnamic acids.

Results from quantification applied to one roasted coffee sample are shown in Table 3.

Table 3
Phenolic Acids Content in Roasted Coffee Sample*

	Arabica coffee sample **g/Kg ± SD**
Caffeic acid	0.367 ± 0.020
p-coumaric acid	0.178 ± 0.005
Ferulic acid	1.641 ± 0.012
o-coumaric acid	0.094 ± 0.014

*Value are expressed as mean ±SD of three determinations

Suitability of the Method

Throughout the study, suitability of the chromatographic system was monitored on roasted coffee sample. The chromatographic parameters such as resolution, selectivity where satisfactory for this compounds. The calculated resolution were not less than 2.47 and the selectivity were not less than 1.514.

Precision

The precision of the analytical method was evaluated by measuring the peak chromatographic area of caffeic acid, 10 times on the same sample. The coefficient of variation (RSD %) was 0.71%.

Conclusion

The developed method is suitable for the identification and separation of caffeic acid, p-coumaric acid, ferulic acid and o-coumaric acid in roasted coffee samples. The results obtain suggest that this HPLC method could be considered as good alternative to already existing methods for the analysis of phenolic acids in coffee. This method may be useful to differentiate and to characterize the types of coffee according to their geographical origin.

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Metodă HPLC pentru identificarea şi separarea unor acizi fenolici din cafea

Rezumat

A fost dezvoltată o metodă HPLC simplă şi reproductibilă pentru determinarea acidului cafeic, acidului p-cumaric, acidului ferulic şi a acidului o-cumaric din probe de cafea prăjită. Acizii fenolici au fost separaţi utilizând o coloană Kromasil 100-5C18 şi eluţie în gradient cu un debit de 0.9 ml min^{-1}*. Probele au fost înregistrate la 320 nm pentru toţi acizii fenolici utilizând un detector de tip diode-array. Liniaritatea, selectivitatea, parametrii de performantă ai sistemului, precizia au valori acceptabile. Această metodă poate fi utilă pentru diferenţierea tipurilor de cafea şi pentru caracterizarea acestora în funcţie de originea lor geografică.*